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Deconjugation of Bile Acids by Intestinal Lactobacilli[1]

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Lactobacillus species normally found in the intestinal tract of humans varied in the ability to deconjugate bile acids, whereas laboratory strains of *Lactobacillus acidophilus* deconjugated both glycocholate and taurocholate. All isolates of *L. acidophilus* from human feces deconjugated taurocholate, whereas only one of six deconjugated glycocholate. None of 13 isolates identified as *L. casei* deconjugated taurocholate, whereas 9 deconjugated glycocholate. The deconjugating system of *L. acidophilus* appeared to be constitutive, required low oxidation-reduction potential, and was most active at pH 6. No degradation beyond deconjugation was detected.

Bacterial modification of primary bile acids contributes to the maintenance of enterohepatic circulation of bile acids in normal humans (6, 14). One of the first major reactions in the catabolism of bile acids by microorganisms is the deconjugation of conjugated bile acids (2). Deconjugation occurs regularly during the enterohepatic circulation in normal humans (6). Reviews by Shimada et al. (20) and Lewis and Gorbach (12) indicate that a number of species of bacteria normally found in the gastrointestinal tract can deconjugate bile acids. The majority of the bacteria capable of deconjugation were strict anaerobes. Hill and Drasar (9) and Aries et al. (1) reported that *Lactobacillus* species isolated from human feces were unable to deconjugate taurocholic acid. Midtvedt and Norman (15) reported that *Lactobacillus arabinosus* deconjugated both taurocholic and glycocholic acids, whereas *L. brevis* deconjugated only glycocholic acid. *L. acidophilus, L. casei,* and *L. delbrueckii* did not deconjugate either of the two bile acids. Floch et al. (5) isolated *Lactobacillus* species from human feces which deconjugated glycocholic, glycodexycholic, and glycochenodeoxycholic acids. The species of lactobacillus were not identified.

Preliminary studies in our laboratory indicated that *L. acidophilus* deconjugated taurocholic acid. The purpose of this investigation was to study factors affecting deconjugation and to determine how widespread the activity is in *Lactobacillus* species isolated from human feces.

MATERIALS AND METHODS

Source and maintenance of cultures. *L. acidophilus* NCFM, ATCC 4962, and CNRZ 216 were from the culture collection in the Food Science Department of North Carolina State University. Other lactobacilli were isolated from human feces plated on LBS agar (BBL) incubated in a CO_2 atmosphere as described by Gilliland et al. (7). Identification of the isolates was based on characteristics reported by Rogosa and Sharp (19). All cultures were maintained in MRS broth (Difco) using 1% inocula and incubation at 37°C for 18 to 24 h. They were stored at 5°C between transfers.

Detection of deconjugation. Cultures were grown in MRS broth containing 10^{-3} M sodium taurocholate (Calbiochem). In most experiments filter-sterilized (0.45-μm membrane filter; Millipore Corp.) solutions of the bile salts were added to autoclaved broth. However, in some experiments the bile salts were added to broth prior to autoclaving without affecting the experimental results. The broth samples inoculated (1%) with fresh MRS broth cultures of lactobacilli were incubated for 24 h at 37°C aerobically or anaerobically (GasPak system from BBL). In some experiments 0.2% sodium thioglycolate was added to the MRS broth (MRS Thio broth) prior to autoclaving to provide low oxidation-reduction (O/R) conditions without using the GasPak system. After incubation, the samples (15 ml each) were adjusted to pH 7 with 1 N NaOH and centrifuged for 10 min at 12,000 × *g* to remove the cells. The supernatant fraction was adjusted to pH 1 with 1 N HCl. The free bile acids were extracted with three 50-ml portions of ethyl acetate (16). The residue was further extracted with three 50-ml portions of *n*-butanol to recover the conjugated bile acids (16). The extracts were evaporated to dryness on a rotary evaporator under reduced pressure. The residues were dissolved in 15 ml of 0.01 N NaOH. The concentrations of free and conjugated bile salts in the samples were determined colorimetrically by the method of Irvin et al. (10). The concentration of free bile acids (micromoles per milliliter) was expressed as cholic acid and the conjugated bile acids as taurocholic acid. Uninoculated MRS broth containing 10^{-3} M taurocholate and MRS broth cultures of the lactobacilli were assayed in a similar manner to determine if substances that might interfere with the

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analyses were present. Samples containing glycocholic acid could not be assayed in the above manner since glycocholic acid is soluble in ethyl acetate.

In experiments to screen lactobacilli for deconjugation activity, a shorter method was used. MRS broth (10 ml) containing sodium taurocholate or glycocholate (10^{-3} M) was used for these assays. After incubation (24 h at 37°C in a GasPak system), 5-ml samples were placed in clean screw-cap test tubes. (The remaining 5-ml portions were incubated for an additional 4 days in a GasPak system.) One milliliter of 4 N HCl was added, followed by 6 ml of ethyl acetate. The tubes were shaken vigorously for 1 min, the phases were allowed to separate, and a portion of the ethyl acetate layer was removed for analysis by thin-layer chromatography. If the samples were negative for free bile acids, the analysis was repeated on the samples that had been incubated for 5 days.

Thin-layer chromatography. Samples and appropriate standards were spotted onto 0.25-mm layers of silica gel which had been heat activated at 110°C for 10 min. The chromatograms were developed to a height of 15 cm in glass tanks lined with filter paper saturated with the solvent. The following solvent systems were used: cyclohexane-ethyl acetate-acetic acid (7:23:3) (17); butanol-acetic acid-water (10:1:1) (21); methylene chloride-acetone-acetic acid (7:2:1) (21); and cyclohexane-chloroform-methanol-acetic acid (20:60:10:1) (21). After drying, the developed plates were sprayed with vanillin-sulfuric acid reagent and heated at 120°C as described by Stahl (21).

Assays using nongrowing cultures. *L. acidophilus* NCFM was grown in MRS Thio broth with or without 10^{-3} M sodium taurocholate. The cells were harvested by centrifugation and resuspended to the desired concentration in sugar-free MRS Thio broth (the glucose and beef extract had been deleted) plus sodium taurocholate (10^{-3} M). In experiments conducted to determine optimum pH, samples of the mixture were adjusted to pH 5, 6, 7, and 8 using either 1 N HCl or 1 N NaOH. Samples were extracted and analyzed colorimetrically for free bile salts after selected incubation (37°C) times.

RESULTS

L. acidophilus deconjugated sodium taurocholate when growing in MRS broth in a GasPak system (Table 1). The appearance of free bile acids in the cultured samples was accompanied in each case by a disappearance of conjugated bile acids. Strain 4962 appeared to be more active than strain NCFM. However, the increased deconjugation was probably due to the fact that a higher population was present in the strain 4962 culture at the time of assay. The plate counts (MRS broth plus 1.5% agar) were 4.8×10^8 and 1.3×10^9/ml for strains NCFM and 4962, respectively.

Results presented in Table 2 emphasize the necessity of a low O/R potential for the deconjugation activity. Essentially no free bile acid was detected in the aerobic sample. Growth in the sample containing thioglycolate resulted in more deconjugation than in the sample in the GasPak system. This may have been due to a lower initial O/R potential in the MRS Thio broth.

Experiments were conducted to compare the activity of cells grown in the presence of sodium taurocholate to those grown without the bile salt. (Sodium taurocholate had no apparent effect on growth of the culture.) The cells were harvested from the growth media and added (approximately 3×10^9/ml) to sugar-free MRS Thio broth containing 10^{-3} M sodium taurocholate. The samples were assayed for free bile salts after 18 h of incubation (37°C) in a GasPak system. Results from a representative experiment (Table 3) revealed about the same amount of free bile acids in both samples.

The deconjugating system in *L. acidophilus* NCFM was most active near pH 6 (Fig. 1). Each point on this curve represents the average from two experiments (the results from both experiments were very similar) in which deconjugation was measured using nongrowing cells in sugar-free MRS Thio broth.

Lactobacilli isolated from human feces varied in the ability to deconjugate bile salts. Some failed to deconjugate either taurocholate or glycocholate, some deconjugated only one of the two, and some were active on both (Table 4). All six isolates identified as *L. acidophilus* de-

TABLE 1. *Deconjugation of sodium taurocholate by L. acidophilus*

Sample	Bile salts (μmol/ml)	
	Free[a]	Conjugated[a]
Control	0.12	1.38
L. acidophilus NCFM	0.73	0.65
L. acidophilus 4962	1.30	0.42

[a] Twenty-four hours of incubation at 37°C in a GasPak system.

TABLE 2. *Effect of aerobic growth on deconjugation of sodium taurocholate by L. acidophilus NCFM*

Sample	Free bile acid (μmol/ml)[a]
Aerobic[b]	0.09
GasPak[b]	0.25
Thioglycolate[c]	0.44

[a] Measured after 18 h of incubation at 37°C.
[b] MRS broth plus 10^{-3} M taurocholate.
[c] MRS Thio broth plus 10^{-3} M taurocholate.

TABLE 3. *Effect of prior growth in the presence of sodium taurocholate on deconjugating activity of cells of L. acidophilus NCFM*

Growth medium	Free bile salts[a] (μmol/ml)
MRS	0.57
MRS + 10^{-3} M taurocholate	0.55

[a] Measured after 18 h of incubation at 37°C in a GasPak system.

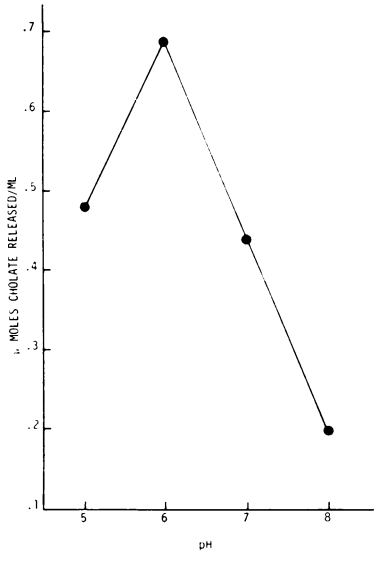

FIG. 1. *Optimum pH for deconjugation of sodium taurocholate by L. acidophilus NCFM. Cells of L. acidophilus in sugar-free MRS Thio broth plus 10^{-3} M taurocholate; assayed for free bile acids after 5 h of incubation.*

conjugated taurocholate, but only one of the six deconjugated glycocholate. The laboratory strains NCFM, 4962, and 216 (results not shown) deconjugated both bile salts. None of 13 isolates identified as *L. casei* deconjugated sodium taurocholate, whereas 9 of the 13 deconjugated glycocholate. Except for *L. buchneri*, all other species tested included some isolates that exhibited deconjugation activity. However, two isolates of *L. fermenti*, one of *L. leichmanni*, and one of *L. plantarum* did not deconjugate either taurocholate or glycocholate. One of the two isolates of *L. salivarius* deconjugated taurocholate and the other deconjugated glycocholate.

Thin-layer chromatographic analyses were done on most of the samples throughout all experiments in this study. Analyses of the free bile salts fractions, using several solvent systems, revealed that cholic acid was the only free bile acid present. This result indicates that no degradation occurred beyond deconjugation.

DISCUSSION

Most species of lactobacilli isolated from human feces deconjugated sodium taurocholate and/or glycocholate. Some reports (1, 9) indicate that fecal lactobacilli do not possess this ability, whereas results reported by others (5, 15) vary. These differences may have resulted from variations in growth conditions, conjugated bile acid used, or assay procedures. Hill and Drasar (9) indicated that bacteria capable of deconjugating taurocholate were also active on glycocholate. Such does not appear to be the case with intestinal lactobacilli. Most *L. acidophilus* isolates deconjugated taurocholate and not glycocholate. On the other hand, none of the *L. casei* were active on taurocholate whereas most were active on glycocholate.

The deconjugation of bile salts by *L. acidophilus* requires a low O/R potential. The greater amount of deconjugation in the MRS Thio broth than in the MRS broth in a GasPak system may have resulted from the O/R potential of the MRS Thio being lower initially. The reduction of O/R potential in the MRS in the GasPak system would require time for equilibration as the system became anaerobic. The pH optimum (pH 6.0) for *L. acidophilus* NCFM appears to be within the range reported for other bacterial deconjugation systems (2, 9). Resting cells of *L. acidophilus* harvested from broth containing no taurocholate were as active with regard to deconjugation as were cells that had been grown in broth containing taurocholate, indicating that the deconjugating system is consti-

TABLE 4. *Deconjugation of sodium taurocholate and sodium glycocholate by lactobacilli isolated from human feces*

Species[b]	Deconjugation[a]	
	Taurocholate	Glycocholate
L. acidophilus	6/6[c]	1/6
L. buchneri	0/3	0/3
L. casei	0/13	9/13
L. fermenti	1/5	3/5
L. leichmanni	3/4	3/4
L. plantarum	0/3	2/3
L. salivarius	1/2	1/2

[a] Cultures were grown in MRS plus bile salts in a GasPak system and were checked at 1 and 5 days by extracting free bile salts and assaying by thin-layer chromatography.

[b] All species listed were isolated from human feces.

[c] Number positive/number tested for each species.

tutive in *L. acidophilus*. Any benefit these organisms might derive from deconjugating bile acids is not clear. Since the cholate is apparently not further metabolized, the organism must not use the steroid moiety. No attempt was made to determine the fate of the taurine or glycine. The possibility exists that the deconjugation was due to the action of an enzyme system that has another function within the bacterial cell.

The importance of most of the metabolites resulting from the bacterial action on bile salts in the intestinal tract is unknown (13). This is especially true in normal subjects. Conventionally reared animals eliminate more bile acids through feces than do germfree animals (4, 11, 12). All the excreted bile acids in the conventional animals are deconjugated. Eyssen (4) has suggested that the increased excretion of bile acids can lead to a faster rate of catabolism of cholesterol to bile acids. Several reports (3, 8, 11) have indicated that establishment of deconjugating bacteria in the intestine of germfree animals did not result in total deconjugation of bile acids excreted in feces, nor was there a significant increase in the total amounts of bile acids excreted. This finding may have been due to the use of inappropriate strains of bacteria or to the possibility that total conditions of the digestive tract were not comparable to that of the conventional animal (3). It is likely that more than just a few strains of bacteria are involved in the conventional animal.

Another area where deconjugation may be important is in the ecological control of microorganisms in the intestinal tract. Deconjugated bile acids are more inhibitory to bacteria than are conjugated ones (5, 18); therefore, deconjugation of bile acids may enhance antagonistic actions of intestinal lactobacilli toward intestinal pathogens, thus helping to maintain a favorable balance among species present in the intestinal tract.

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